REFERENCE

P14

Cloning and expression of carbohydrate binding module in *Pichia pastoris*

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The main goal of this work is the production of recombinant biologically active peptides fused with a Carbohydrate Binding Module (CBM). Aiming at the optimization of large scale expression, CBM peptide production was done by cloning CBM coding sequence in two different systems of *Pichia pastoris*: pGAPZαC which has a constitutive promoter and pPICZαC which has an inductive promoter. The integration of the CBM coding sequence in the yeast genome was confirmed by slot-blot for both expression systems. Transcription was analysed by northern-blot and SDS-PAGE. The results obtained with these two expression systems were different. Apparently, there were no clones of *P. pastoris* transformed with pGAPZαC-CBM that had produced any protein with starch affinity, under the batch and fed-batch conditions tested in this work. On the other hand, only one studied clone of *P. pastoris* transformed with pPICZαC-CBM vector had produced, in batch conditions, a protein with affinity for starch. However, under fed-batch conditions, the results obtained with this clone were not conclusive, suggesting that conditions for large scale production must be optimized.